Location analysis of estrogen receptor α target promoters reveals that FOXA1 defines a domain of the estrogen response

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Nuclear receptors can activate diverse biological pathways within a target cell in response to their cognate ligands, but how this compartmentalization is achieved at the level of gene regulation is poorly understood. We used a genome-wide analysis of promoter occupancy by the estrogen receptor α (ERα) in MCF-7 cells to investigate the molecular mechanisms underlying the action of 17β-estradiol (E_2) in controlling the growth of breast cancer cells. We identified 153 promoters bound by ERα in the presence of E_2. Motif-finding algorithms demonstrated that the estrogen response element (ERE) is the most common motif present in these promoters whereas conventional chromatin immunoprecipitation assays showed E_2-modulated recruitment of coactivator AIB1 and RNA polymerase II at these loci. The promoters were linked to known ERα targets but also to many genes not directly associated with the estrogenic response, including the transcriptional factor FOXA1, whose expression correlates with the presence of ERα in breast tumors. We found that ablation of FOXA1 expression in MCF-7 cells suppressed ERα binding to the prototypic *TFF1* promoter (which contains a FOXA1 binding site), hindered the induction of *TFF1* expression by E_2, and prevented hormone-induced reentry into the cell cycle. Taken together, these results define a paradigm for estrogen action in breast cancer cells and suggest that regulation of gene expression by nuclear receptors can be compartmentalized into unique transcriptional domains by means of licensing of their activity to cofactors such as FOXA1.

ChIP-on-chip | forkhead box | transcription | cell cycle

Estradiol (17β-estradiol, E_2) is a potent growth factor of human breast cancer cells that exerts its action mainly through estrogen receptor α (NR3A1, ERα), a member of the superfamily of nuclear receptors (1). Despite significant advancement into our understanding of the molecular mechanisms of ERα action (2), little is known about mediators of the estrogen pathway that assist in the initiation, compartmentalization, and propagation of its signal at the level of gene expression. Delineation of how ERα induces precise biological responses in breast cancer cells and other cell types has clearly been limited by the lack of data on the transcriptional regulatory regions of ERα direct target genes.

ERα regulates the expression of target genes by binding to specific sites in the chromatin, referred to as estrogen response elements (EREs) (3), or by interacting with other transcription factors bound to their own specific recognition sites (4–6). Determination of ERα target genes has recently been undertaken by using DNA microarrays, identifying hundreds of genes with altered expression upon E_2 treatment of human breast cancer cells (7–17). However, while providing information of the global action of E_2 in these cells, gene expression profiling can rarely discriminate between direct and indirect ERα targets. In addition, bioinformatic and comparative genomics have also been used successfully to identify high-affinity and physiologically relevant EREs encoded in the human genome (18, 19). These studies have also some constraints, including their limitation to consensus EREs and the general absence of large scale functional data linking these putative binding sites with gene expression in specific cell types.

Recently, chromatin immunoprecipitation (ChIP) has been used in combination with promoter or genomic DNA microarrays to identify loci recognized by transcription factors in a genome-wide manner in mammalian cells (20–24). This technology, termed ChIP-on-chip or location analysis, can therefore be used to determine the global gene expression program that characterize the action of a nuclear receptor in response to its natural ligand. For this study, we first constructed a human proximal promoter DNA microarray containing ≈19,000 promoters and then monitored occupancy by ERα at these promoters in MCF-7 breast cancer cells in the presence of E_2. Our experiments identified genes that include known ERα targets, genes previously associated with the E_2 response but not characterized as direct targets, and several novel target genes. Among those genes, we identified the transcriptional factor FOXA1, whose expression correlates with the presence of ERα in breast tumors. We found that knock-down of FOXA1 expression in MCF-7 in cells using small interfering RNA (siRNA) depletion experiments diminished ERα binding to the prototypic *TFF1* promoter (which contains a FOXA1-binding site), reduced the induction of *TFF1* expression by E_2, and prevented hormone-induced reentry into the cell cycle. Our results demonstrate that FOXA1 licensing plays an unsuspected role in defining a subdomain of the transcriptional response to E_2 in breast cancer cells, and suggest that more precise therapeutic approaches could be developed to target the wide-ranging action of E_2 in the normal and disease states.

Materials and Methods

Human Promoter Microarray Design. The strategy adopted to design our promoter microarray is similar to the one used by the Young group (22). Full-length complementary DNAs were extracted from Reference Sequence (Refseq) and Mammalian Gene Collection (MGC) databases and filtered to eliminate redundancy and incomplete cDNAs. Their transcription start sites were then located by using the University of California at Santa Cruz (UCSC) genome browser (25), and the sequence ranging from 800 bp upstream of the transcription start sites to 200 bp downstream of the transcription start sites was extracted by using the UCSC database assemblage July 2003 (25). Primer pairs were designed by using the Primer3 algorithm (26), and the specificity was tested *in silico* by using a virtual PCR algorithm (27). When the primer pair gave no satisfactory virtual PCR results, a new primer pair was designed by using Primer3 and tested again. The process was iterated three

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Abbreviations: ChIP, chromatin immunoprecipitation; ERα, estrogen receptor α; E_2, 17β-estradiol; ERE, estrogen response element; siRNA, small interfering RNA.

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times to generate primer pairs predicted to be efficient to amplify promoter regions from human genomic DNA for almost all of our selected genes. This strategy was adopted after preliminary results showed that a simpler primer design approach did not generate good results when we tried to amplify promoter regions from human genomic DNA. This primer design pipeline allowed us to design primer pairs to amplify promoter regions from human genomic DNA with a success rate of ≈80%, which is slightly better than that reported previously (22). At the date of the download (July 2004) 21,416 RefSeq and 16,521 MGC entries were retrieved. After the filtering process, 18,741 of them were selected and submitted to primer design. Primers were obtained for 18,660 promoters, and 188 controls were added (located in exons and far from any known genes).

Genome-Wide Location Analysis and ChIP. After 72 h of steroid deprivation followed by 45 min of E_2 (100 nM) treatment, MCF-7 cells were fixed with 1% final concentration formaldehyde for 10 min at room temperature, rinsed with 1× PBS, and harvested. The resultant cell pellet was lysed and sonicated, and protein–DNA complexes were enriched by immunoprecipitation with the ERα-specific antibody (Santa Cruz Biotechnology); beads were added and washed as described (28). After de-crosslinking, the enriched DNA was repaired with T4 DNA polymerase (New England Biolabs) and ligated with linkers, as described in ref. 22. DNA was amplified by using ligation-mediated PCR (LM-PCR), and then fluorescently labeled by using BioPrime Array CGH genomic labeling kit and the Cy5 fluorophore (Invitrogen). A sample of DNA that had not been enriched by immunoprecipitation was subjected to LM-PCR and labeled with Cy3 fluorophore. Both IP-enriched and nonenriched pools of labeled DNA were hybridized to the human promoter array described above. The *P* value threshold used to select target promoters for further analyses was determined empirically by testing randomly selected targets by standard ChIP/quantitative PCR. Based on these experiments, we used $P = 0.005$ because our estimated false-positive rate was <10% (genes tested = 34, see Table 2, which is published as supporting information on the PNAS web site) using this threshold. FOXA1 ChIP assays were performed by using two distinct antibodies from Chemicon and Santa Cruz Biotechnology. RNA polymerase II and AIB1 ChIP assays were performed by using antibodies from Upstate Biotechnology (Lake Placid, NY) and Santa Cruz Biotechnology, respectively.

Promoter Sequence Analysis. We used a motif-finding algorithm (MDScan) (29) to uncover motifs that are highly represented in our set of promoter sequences. The presence of EREs and FOXA1-binding sites was also determined by using MACVECTOR (Accelrys, San Diego) and TRANSFAC (30). The logo pictured in Fig. 1*A* was generated by using WEBLOGO (weblogo.berkeley.edu/logo.cgi).

Functional Classification of Target Genes. Functional categories were assigned by using both GO (www.fatigo.org) and manual inspection by using PubMed (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = PubMed).

Cell Culture, Luciferase, and Cell Cycle Entry Assays. MCF-7 cells were cultured as described (28). For the luciferase assay, cells were transfected with Lipofectamine 2000 (Invitrogen) with 0.4 μg of TFF1-Luc (31) and 0.2 μg of pCMVβGal internal control per well, 0.1 μg of CMX-ERα, and 100 nM final concentration of FOXA1 or control siRNA (SMARTpool reagents, Dharmacon Research, Lafayette, CO). Twelve hours after transfection, fresh medium was added, incubated for 12 h, and then treated with ethanol (vehicle) or E_2 (10^{-7} M) for 20 h. Cells were then harvested and assayed for luciferase and β-galactosidase activities. For FACS analysis, cells were cultured in steroid-deprived media for 48 h, transfected with *FOXA1* or control siRNAs, and incubated for 36 h and treated with

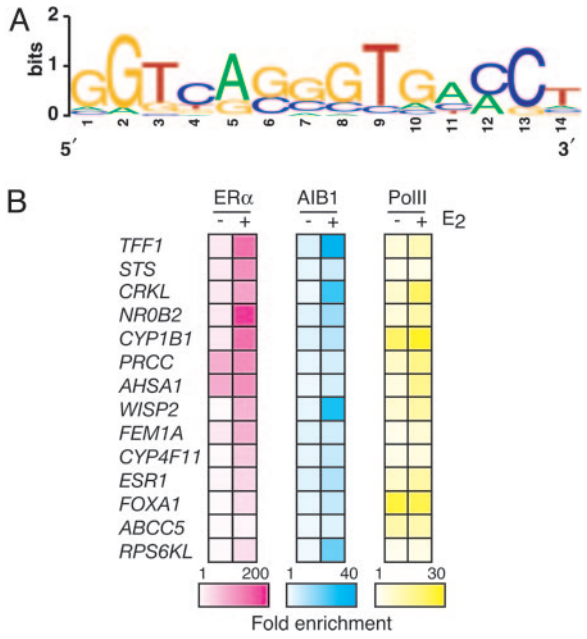

Fig. 1. Genome-wide location analysis of direct ERα transcriptional targets in MCF-7 breast cancer cells. (*A*) Motif-finding algorithms identify the consensus ERE (GGTCANNNTGACCT) as the most common transcription factor-binding motif present in the promoters bound by ERα in the promoter array. The motif was present in 60% of the promoters used for the analysis. (*B*) E_2-modulated recruitment of ERα, the coactivator AIB1, and RNA polymerase II at selected ERα targets in MCF-7 cells as assayed by conventional ChIP.

E_2 or vehicle for 20 h. Cells were then trypsinized, fixed in 70% EtOH, and stored at −20°C overnight. Before analysis, cells were washed in PBS, resuspended in a solution containing 0.5 mg/ml RNase (Sigma) and 5 μg/ml of propidium iodide (Sigma) and analyzed on a FACScan (Becton Dickinson).

Western Blot and RT-PCR. Western blot was performed by using FOXA1 and actin antibodies (Santa Cruz Biotechnology). RT-PCR was conducted as described in ref. 28.

Results and Discussion

ChIP-on-Chip Analysis of ERα Binding. The MCF-7 cell line is a well established model for the study of E_2-induced human breast cancer cell growth and was thus selected for this study (32). To identify targets of ERα in an unbiased genome-wide manner, we constructed a genomic DNA microarray containing the region spanning 800 bp upstream and 200 bp downstream of transcription start sites of 18,660 human genes. We identified a total of 153 promoters ($P < 0.005$) bound by ERα in the presence of E_2 (Table 1 and Table 3, which is published as supporting information on the PNAS web site). We confirmed binding by ERα to a subset of targets by using conventional ChIP assays and quantitative PCR and determined that our rate of false positives was <10% when previously established threshold criteria were used (see *Materials and Methods*). The results of the genome location experiment were further validated by using a motif-finding algorithm that examines the ChIP-on-chip selected sequences and searches for DNA sequence motifs representing the protein–DNA interaction sites (29). The consensus sequence derived from the most frequent motifs found in the ERα-bound promoters corresponds to a perfect estrogen response element (GGTCANNNTGACCT, Fig. 1*A*). If these genes are indeed regulated by E_2-bound ERα, coregulator proteins and RNA polymerase II should also be recruited to the promoters in response to E_2. Examination of a subset of ERα-bound promoters using conventional ChIP demonstrated that a number of loci recruited

Table 1. Functional classification of target genes bound by ERα in MCF-7 cells in the presence of estradiol

Gene	Description
Apoptosis	
CASP7	Caspase 7
IKBKG	Inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ
Carbohydrate metabolism	
GLT25D2	Glycosyltransferase 25 domain-containing 2
HK1	Hexokinase 1
MDH1	Malate dehydrogenase 1, NAD
Cell adhesion	
ANXA6	Annexin A6
ANXA9	Annexin A9
COL5A3	Collagen, type V, α3
NINJ2	Ninjurin 2
Cell-cell signaling	
CTNNBIP1	Catenin, β interacting protein 1
SEMA3B	Sema domain, Ig domain, short basic domain, secreted, (semaphorin)
WISP2	WNT1 inducible signaling pathway protein 2
WNT16	Wingless-type MMTV integration site family, member 16
Cell growth/maintenance	
CHPT1	Choline phosphotransferase 1
EPS8	Epidermal growth factor receptor pathway substrate 8
PRCC	Papillary renal cell carcinoma
SEL1L	Sel-1 suppressor of lin-12-like (*C. elegans*)
TBC1D3	TBC1 domain family, member 3
Cell motility	
CRKL	v-crk sarcoma virus CT10 oncogene homolog
Cell cycle	
ARKRD15	Ankyrin repeat domain 15
BANP	BTG3 associated nuclear protein
CDK5	Cyclin-dependent kinase 5
RBL2	Retinoblastoma-like 2 (p130)
TUSC4	Tumor suppressor candidate 4
Chromosome biogenesis	
SMYD3	SET and MYND domain-containing 3
Co-enzyme metabolism	
COQ4	Coenzyme Q4 homolog (yeast)
MOCS2	Molybdenum cofactor synthesis 2
Cytoskeleton	
FGD3	FYVE, RhoGEF, and PH domain-containing 3
KRT13	Keratin 13
SPTBN4	Spectrin, β, non-erythrocytic 4
TTID	Titin immunoglobulin domain protein (myotilin)
TNS	Tensin
Defense response	
LY6E	Lymphocyte antigen 6 complex, locus E
PGLYRP2	Peptidoglycan recognition protein 2
TFF1	Trefoil factor 1
TFF3	Trefoil factor 3
DNA repair	
RECQL4	RecQ protein-like 4
Immune response	
IL-20	IL-20
Lipid metabolism	
ALDH3B2	Aldehyde dehydrogenase 3 family, member B2
PAFAH2	Platelet-activating factor acetylhydrolase 2, 40 kDa
PCYTIA	Phosphate cytidylyltransferase 1, choline, α isoform
Protein metabolism and modification	
AHSA1	HA1, activator of heat shock 90-kDa protein ATPase homolog 1
B3Gn-T6	β-1,3-*N*-acetylglucosaminyl transferase protein
CST5	Cystatin D
FBXO33	F-box protein 33
H11	Protein kinase H11
HSPH1	Heat shock 105-dKa/110-kDa protein 1
PKIB	Protein kinase (cAMP-dependent, catalytic) inhibitor β
RPS6KL1	Ribosomal protein S6 kinase-like 1
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
TMPRSS3	Transmembrane protease, serine 3
RNA processing	
DDX23	DEAD (Asp-Glu-Ala-Asp) box polypeptide 23
PRPF31	Pre-mRNA processing factor 31 homolog (yeast)
QTRTD1	Queuine tRNA-ribosyltransferase domain-containing 1
THOC3	THO complex 3 Signal transduction
P2RY6	Pyrimidinergic receptor P2Y, G protein-coupled, 6
Steroid and drug metabolism	
BAAT	Bile acid CoA:amino acid *N*-acyltransferase (glycine *N*-choloyltransferase)
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1
CYP4F3	Cytochrome P450, family 4, subfamily F, polypeptide 3
CYP4F11	Cytochrome P450, family 4, subfamily F, polypeptide 11
STS	Steroid sulfatase, arylsulfatase C, isozyme S
UGT2B15	UDP glycosyltransferase 2 family, polypeptide B15
UGT2B17	UDP glycosyltransferase 2 family, polypeptide B17
Transcriptional regulator	
CARP	Cardiac ankyrin repeat protein
ESR1	Estrogen receptor 1
FLJ20097	Hypothetical protein FLJ20097
FOXA1	Forkhead box A1
NR0B2	Nuclear receptor subfamily 0, group B, member 2
PHF15	PHD finger protein 15
PPRC1	PPAR, γ, coactivator-related 1
PROP1	Prophet of Pit1, paired-like Hox transcription factor
TRIM16	Tripartite motif-containing 16
ZNF140	Zinc finger protein 140
ZNF302	Zinc finger protein 302
ZNF485	Zinc finger protein 485
Transport	
ABCA3	ATP-binding cassette, sub-family A (ABC1), member 3
ABCC5	ATP-binding cassette, sub-family C (CFTR/MRP), member
ABCC11	ATP-binding cassette, sub-family C (CFTR/MRP), member
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2
DSCR3	Down syndrome critical region gene 3
NDUFA2	NADH dehydrogenase 1 α subcomplex, 2
NDUFB9	NADH dehydrogenase 1 β subcomplex, 9
P2RX7	Purinergic receptor P2X, ligand-gated ion channel, 7
PDZK1	PDZ domain-containing 1
PKD212	Polycystic kidney disease 2-like 2
RAB7L1	RAB7, member RAS oncogene family-like 1
SLC7A3	Solute carrier family 7, member 3
SLC9A8	Solute carrier family 9 (sodium/hydrogen exchanger), isoform
SLC25A36	Solute carrier family 25, member 36
SLC27A2	Solute carrier family 27, member 2
SYT12	Synaptotagmin XII
UCRC	Ubiquinol-cytochrome *c* reductase complex (7.2 kDa)
ZFYVE1	Zinc finger, FYVE domain-containing

Genes without an assigned function at this level of analysis: C9orf11, C14orf61, C14orf133, C20orf172, CBWD2, CHD1L, CYB561D2, DKFZp434B1272, DKFZp547E1912, DKFZP5641122, DKFZP566J2046, DNC12, DOC1, Eny2, FAHD1, FAM3C, FEM1A, FLJ10871, FLJ11267, FLJ13710, FLJ20094, FLJ20772, FLJ31882, FLJ33761, FLJ33868, GREB1, HAGH, HSPC138, IGSF3, INVS, KIAA1536, KSP37, LOC90668, LOC114926, MDH1, MDS025, MGC8902, MGC10200, MGC11242, MGC26694, MGC35361, MGC47799, MR-1, MSMB, NALP6, NAV3, NUDCD1, PRUNE, RGN, S100A10, SCGB1D2, SMAP, SMILE, TFPT, TRIM51, TSNAX1P1, TSSC4, VEPH1, Y1F1B, ZMAT5. In the case that one locus could be assigned to two distinct genes, both genes were included in the analysis.

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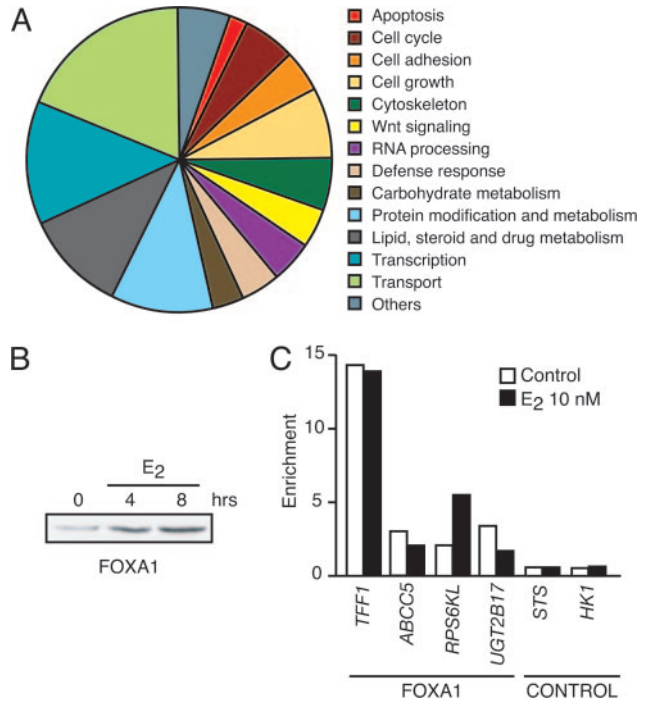

Fig. 2. FOXA1, a target of ERα, is recruited to a subset of ERα targets. (*A*) Pie chart representing major biological functions and processes associated with ERα targets (153) enriched in E_2-treated MCF-7 cells. (*B*) Induction of FOXA1 expression by E_2 as monitored by Western blot. (*C*) FOXA1 recruitment to a subset of ERα-bound promoters containing FOXA1 binding sites as assayed by conventional ChIP. The *STS* and *HK1* promoters serve as a ERα-bound control promoters without a FOXA1-binding site. The results presented are from a single experiment representative of three independent experiments.

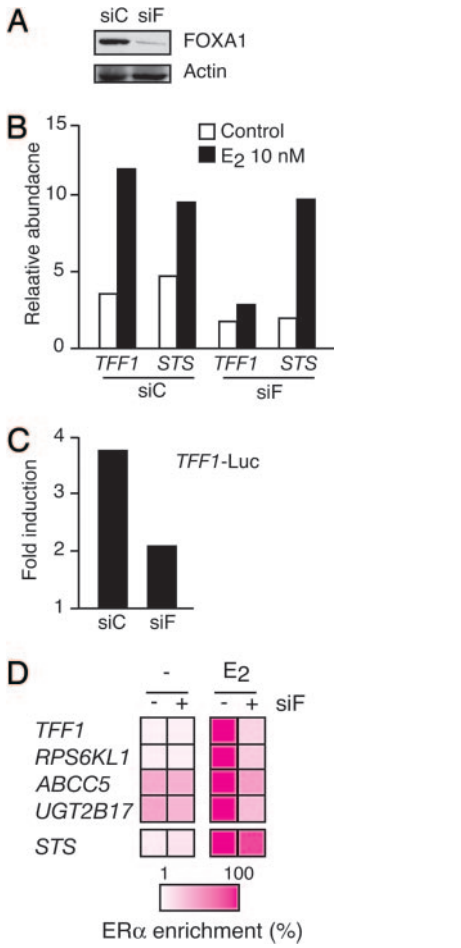

Fig. 3. FOXA1 is required for ERα activity on a subset of target promoters. (*A*) FOXA1 expression in MCF-7 cells transfected with control (siC) and FOXA1 (siF) siRNAs. Actin levels serve as a control for specificity and gel loading. (*B*) FOXA1 is required for the E_2 regulation of *TFF1* expression in MCF-7 cells. RT-PCR analysis of *TFF1* expression was performed with extracts obtained from cells transfected with control (siC) and FOXA1 (siF) siRNAs in the presence or absence of E_2. The *STS* promoter serves as an ERα-bound control promoter without a FOXA1 binding site. (*C*) Knock-down of FOXA1 expression decreases the ability of ERα to stimulate transcription from the *TFF1* promoter. MCF-7 cells were cotransfected with ERα, the TFF1-Luc reporter, and control (siC) or FOXA1 (siF) siRNAs in the presence or absence of E_2. (*D*) FOXA1 is required for E_2-induced recruitment of ERα to the *TFF1*, *RPS6KL1*, *ABCC5*, and *UGT2B17* promoters as assayed by conventional ChIP. The *STS* promoter acts as a control as described in *B*. The cells were treated with vehicle (C) or 100 nM E_2. Results are expressed as the percentage of maximal ERα binding observed in the presence of E_2. For panel *A*, *B* and *C*, the results presented are from a single experiment representative of at least two independent experiments.

the nuclear receptor coactivator AIB1 (also known as SRC-3, pCIP, and ACTR) (33–35) in the presence of the hormone whereas the amount of RNA polymerase II was consistently increased above the basal level observed for each individual gene (Fig. 1*B*). One exception was for ABCC5, a gene previously found to be down-regulated by E_2 (36), demonstrating that both up- and down-regulated genes can be identified by using the promoter array.

FOXA1, a Target of ERα Coexpressed in Breast Tumors, Is Recruited to a Subset of ERα Targets. Although some known direct targets of ERα were selectively enriched from the chromatin of MCF-7 cells (e.g., *CASP7*, *CYP1B1*, *GREB1*, *LY6E*, *SHP*, *SLC25A36/FLJ10618*, *TFF1*, and *WISP2*), most of the genes identified represent novel primary targets of ERα. We used gene ontology (GO) (37) to classify our ERα targets into functional categories and found that ERα regulates a wide array of cellular processes and molecular functions (Table 1 and Fig. 2*A*). Within these categories, we identified genes involved in Wnt signaling (*WNT16*, *WISP2*, *SEMA3B*, *CTNNBIP1*), steroid metabolism (*CYP1B1*, *STS*, *UGT2B15*, *UGT2B17*), multidrug resistance (*ABCC5*, *ABCC11*), and cell cycle regulation (*CDK5* and *RBL2*, also known as p130). Given the well known property of E_2 to stimulate cell cycle progression of MCF-7 cells and other breast cancer cell lines (38), it was surprising that few key genes known to regulate the cell cycle were obtained in our location analysis. Although some ERα targets are likely to be regulated by means of enhancers located at a great distance form the transcription start sites and be missed by a promoter array, these results do suggest that ERα requires specific downstream effectors to regulate cell growth. These effectors are likely to be involved in transcriptional regulation, and this category was well represented among ERα targets (Fig. 2*A*). In addition to the known regulation by ERα of its own promoter (*ESR1*) and that of the orphan nuclear receptor SHP (*NR0B2*) (39), we identified the nuclear receptor coactivator PRC (*PPRC1*) and the forkhead transcription factor HNF3α/FOXA1 (*FOXA1*) as direct targets of ERα. Interestingly, the expression of *FOXA1*, a pioneer factor with the ability to initiate chromatin opening events (40) and previously shown to establish a promoter environment favorable to transcriptional activation by ERα (41), correlates (Fig. 6, which is published as supporting information on the PNAS web site, $r^2 = 0.7987$) with the presence of ERα in human breast tumors (42, 43) and is rapidly induced by E_2 in MCF-7 cells (Fig. 2*B*). In addition, motif-finding analysis using the consensus FOXA1 binding site WTGRTTNRTT revealed that a specific subset (≈12%) of the ERα-bound promoters contained FOXA1 recognition sites. Conventional ChIP experiments on selected promoter regions detected various levels of

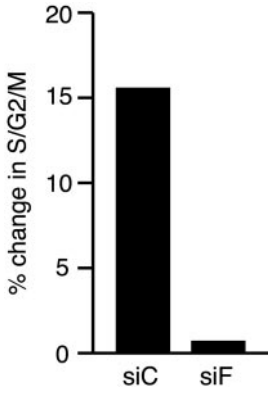

Fig. 4. Effect of FOXA1 knock-down on cell cycle entry in response to E_2. Results shown represent the percentage change in cells in S, G_2, and M phases stimulated by E_2 relative to untreated cells in the presence of control (siC) or FOXA1 (siF) siRNAs. The results presented are from a single experiment representative of two independent experiments.

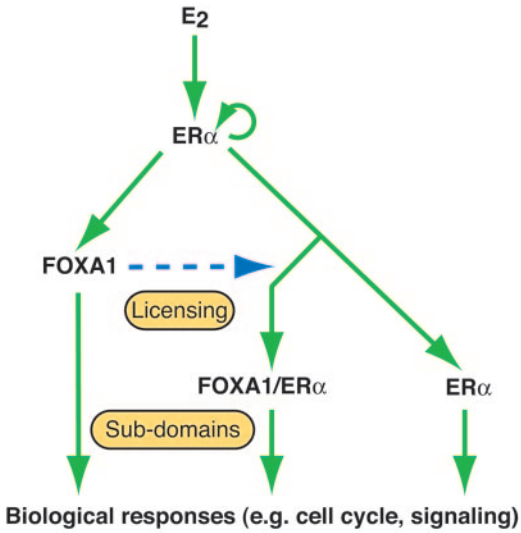

Fig. 5. Model illustrating how FOXA1 licensing defines subdomains of E_2 action in breast cancer cells. Green arrows represent direct transcriptional activity of ERα and FOXA1, and the dashed blue arrow indicates the action of FOXA1 as a modulator of ERα binding to a subset of promoters. The presence of FOXA1 thus grants permission to ERα to regulate a subset of the hormonal response, which can be further amplified by positive regulation of *FOXA1* expression by E_2-bound ERα.

enrichment of these sequences with antibodies against FOXA1 in both the absence or the presence of E_2 (Fig. 2*C*). *TFF1*, a gene also referred to as pS2 and known to be strongly regulated by ERα (44), displayed the most robust enrichment of FOXA1 at its promoter, whereas control promoters without a FOXA1 binding site (*STS* and *HK1*) failed to recruit FOXA1. Taken together, these results suggest that FOXA1 could serve as a licensing factor to propagate a specific domain of the estrogenic response in breast cancer cells.

FOXA1 Is Required for ERα Action on the *TFF1* Promoter. We next examined whether FOXA1 plays a functional role in transcriptional activation of this subset of ERα target genes by transfecting siRNAs directed against *FOXA1* in MCF-7 cells. The presence of the siRNAs specifically knocked-down FOXA1 protein level (Fig. 3*A*) and reduced the ability of E_2 to stimulate the expression of a selected FOXA1/ERα target, *TFF1* (Fig. 3*B*), but not the control promoter *STS*. Similar results were obtained when the ability of ERα to stimulate the activity of the *TFF1* promoter was tested in a cotransfection assay in MCF-7 cells. As shown in Fig. 3*C*, introduction of siRNAs directed against FOXA1 considerably impaired the response of the *TFF1* promoter to E_2. The introduction of siRNA directed against FOXA1 did not affect the expression of ERα as monitored by Western blot (data not shown). Because FOXA1 binding to the *TFF1* promoter was not affected by treatment with E_2 (Fig. 2*B*), we next investigated whether the presence of FOXA1 is required for binding of ERα to the *TFF1* promoter as well as other ERα-bound promoters containing FOXA1 sites. As shown in Fig. 3*D*, knock-down of FOXA1 expression resulted in a marked reduction of the E_2-induced recruitment of ERα to the *TFF1* promoter, as well as to the *RPS6KL1*, *ABCC5*, and *UGT2B17* promoters, whereas the recruitment of ERα to a control promoter (*STS*) was not affected. These results demonstrate that FOXA1 plays an important role in ERα binding and transcriptional activity of a specific subset of FOXA1/ERα target promoters in MCF-7 cells.

FOXA1 Is Required for E_2-Induced Reentry into the Cell Cycle. One hallmark of E_2 action is its ability to induce synchronous cell cycle reentry of steroid-deprived quiescent breast cancer cells (45). We thus tested the possibility that FOXA1 could serve as a mediator of ERα action in this process. MCF-7 cells synchronized in quiescence by depletion of steroid hormones for 48 h were released from quiescence by exposure to E_2 and harvested for cell cycle analysis by flow cytometry. As shown in Fig. 4, MCF-7 cells transfected with siRNAs directed against *FOXA1* failed to reenter the cell cycle upon stimulation with E_2.

Compartmentalization of the Hormonal Response. In this study, using a combination of genome-wide location, genetic analyses, and functional assays, we identified FOXA1 as being essential for ERα binding to *TFF1*, a prototypic gene representing a subset of ERα target promoters, and required for E_2-induced reentry of quiescent breast cancer cells into the cell cycle. These results not only present a paradigm in estrogen action but suggest a mechanism by which nuclear receptors can regulate a specific subset of genes and biological responses with the cooperation of downstream effectors that are essential to both initiate and propagate the hormonal signal (Fig. 5). This study demonstrates that licensing factors, such as FOXA1, that are both under hormonal control and necessary for the hormonal response can be used to compartmentalize the action of nuclear receptors at the level of the genome. These findings thus suggest the existence of new opportunities to target more precisely the action of nuclear receptors for the prevention and management of hormone-dependent diseases.

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1. Osborne, C. K. & Schiff, R. (2005) *J. Clin. Oncol.* **23,** 1616–1622.
2. Smith, C. L. & O'Malley, B. W. (2004) *Endocr. Rev.* **25,** 45–71.
3. O'Lone, R., Frith, M. C., Karlsson, E. K. & Hansen, U. (2004) *Mol. Endocrinol.* **18,** 1859–1875.
4. Herrlich, P. (2001) *Oncogene* **20,** 2465–2475.
5. Pfahl, M. (1993) *Endocr. Rev.* **14,** 651–658.
6. Safe, S. & Kim, K. (2004) *Prog. Nucleic Acid Res. Mol. Biol.* **77,** 1–36.
7. Inoue, A., Yoshida, N., Omoto, Y., Oguchi, S., Yamori, T., Kiyama, R. & Hayashi, S. (2002) *J. Mol. Endocrinol.* **29,** 175–192.
8. Seth, P., Krop, I., Porter, D. & Polyak, K. (2002) *Oncogene* **21,** 836–843.

9. Soulez, M. & Parker, M. G. (2001) *J. Mol. Endocrinol.* **27,** 259–274.
10. Lobenhofer, E. K., Bennett, L., Cable, P. L., Li, L., Bushel, P. R. & Afshari, C. A. (2002) *Mol. Endocrinol.* **16,** 1215–1229.
11. Weisz, A., Basile, W., Scafoglio, C., Altucci, L., Bresciani, F., Facchiano, A., Sismondi, P., Cicatiello, L. & De Bortoli, M. (2004) *J. Cell. Physiol.* **200,** 440–450.
12. Coser, K. R., Chesnes, J., Hur, J., Ray, S., Isselbacher, K. J. & Shioda, T. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 13994–13999.
13. Wang, D. Y., Fulthorpe, R., Liss, S. N. & Edwards, E. A. (2004) *Mol. Endocrinol.* **18,** 402–411.
14. Cunliffe, H. E., Ringner, M., Bilke, S., Walker, R. L., Cheung, J. M., Chen, Y. & Meltzer, P. S. (2003) *Cancer Res.* **63,** 7158–7166.
15. Frasor, J., Danes, J. M., Komm, B., Chang, K. C., Lyttle, C. R. & Katzenellenbogen, B. S. (2003) *Endocrinology* **144,** 4562–4574.
16. Charpentier, A. H., Bednarek, A. K., Daniel, R. L., Hawkins, K. A., Laflin, K. J., Gaddis, S., MacLeod, M. C. & Aldaz, C. M. (2000) *Cancer Res.* **60,** 5977–5983.
17. Lin, C. Y., Strom, A., Vega, V. B., Kong, S. L., Yeo, A. L., Thomsen, J. S., Chan, W. C., Doray, B., Bangarusamy, D. K., Ramasamy, A., *et al.* (2004) *Genome Biol.* **5,** R66.
18. Bourdeau, V., Deschenes, J., Metivier, R., Nagai, Y., Nguyen, D., Bretschneider, N., Gannon, F., White, J. H. & Mader, S. (2004) *Mol. Endocrinol.* **18,** 1411–1427.
19. Kamalakaran, S., Radhakrishnan, S. K. & Beck, W. T. (2005) *J. Biol. Chem.* **280,** 21491–21497.
20. Ren, B., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A. & Dynlacht, B. D. (2002) *Genes Dev.* **16,** 245–256.
21. Weinmann, A. S., Yan, P. S., Oberley, M. J., Huang, T. H. & Farnham, P. J. (2002) *Genes Dev.* **16,** 235–244.
22. Odom, D. T., Zizlsperger, N., Gordon, D. B., Bell, G. W., Rinaldi, N. J., Murray, H. L., Volkert, T. L., Schreiber, J., Rolfe, P. A., Gifford, D. K., *et al.* (2004) *Science* **303,** 1378–1381.
23. Cam, H., Balciunaite, E., Blais, A., Spektor, A., Scarpulla, R. C., Young, R., Kluger, Y. & Dynlacht, B. D. (2004) *Mol. Cell* **16,** 399–411.
24. Cawley, S., Bekiranov, S., Ng, H. H., Kapranov, P., Sekinger, E. A., Kampa, D., Piccolboni, A., Sementchenko, V., Cheng, J., Williams, A. J., *et al.* (2004) *Cell* **116,** 499–509.
25. Karolchik, D., Baertsch, R., Diekhans, M., Furey, T. S., Hinrichs, A., Lu, Y. T., Roskin, K. M., Schwartz, M., Sugnet, C. W., Thomas, D. J., *et al.* (2003) *Nucleic Acids Res.* **31,** 51–54.
26. Rozen, S. & Skaletsky, H. (2000) *Methods Mol. Biol.* **132,** 365–386.
27. Lexa, M., Horak, J. & Brzobohaty, B. (2001) *Bioinformatics* **17,** 192–193.
28. Laganiere, J., Deblois, G. & Giguere, V. (2005) *Mol. Endocrinol.* **19,** 1584–1592.
29. Liu, X. S., Brutlag, D. L. & Liu, J. S. (2002) *Nat. Biotechnol.* **20,** 835–839.
30. Matys, V., Fricke, E., Geffers, R., Gossling, E., Haubrock, M., Hehl, R., Hornischer, K., Karas, D., Kel, A. E., Kel-Margoulis, O. V., *et al.* (2003) *Nucleic Acids Res.* **31,** 374–378.
31. Lu, D., Kiriyama, Y., Lee, K. Y. & Giguère, V. (2001) *Cancer Res.* **61,** 6755–6761.
32. Lacroix, M. & Leclercq, G. (2004) *Breast Cancer Res. Treat.* **83,** 249–289.
33. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X.-Y., Sauter, G., Kallioniemi, O.-P., Trent, J. M. & Meltzer, P. S. (1997) *Science* **277,** 965–968.
34. Chen, H., Lin, R. J., Schlitz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y. & Evans, R. M. (1997) *Cell* **90,** 569–580.
35. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K. & Rosenfeld, M. G. (1997) *Nature* **387,** 677–684.
36. Vendrell, J. A., Magnino, F., Danis, E., Duchesne, M. J., Pinloche, S., Pons, M., Birnbaum, D., Nguyen, C., Theillet, C. & Cohen, P. A. (2004) *J. Mol. Endocrinol.* **32,** 397–414.
37. Al-Shahrour, F., Diaz-Uriarte, R. & Dopazo, J. (2004) *Bioinformatics* **20,** 578–580.
38. Prall, O. W., Rogan, E. M. & Sutherland, R. L. (1998) *J. Steroid Biochem. Mol. Biol.* **65,** 169–174.
39. Lai, K., Harnish, D. C. & Evans, M. J. (2003) *J. Biol. Chem.* **278,** 36418–36429.
40. Cirillo, L. A., Lin, F. R., Cuesta, I., Friedman, D., Jarnik, M. & Zaret, K. S. (2002) *Mol. Cell* **9,** 279–289.
41. Robyr, D., Gegonne, A., Wolffe, A. P. & Wahli, W. (2000) *J. Biol. Chem.* **275,** 28291–28300.
42. van 't Veer, L. J., Dai, H., van de Vijver, M. J., He, Y. D., Hart, A. A., Mao, M., Peterse, H. L., van der Kooy, K., Marton, M. J., Witteveen, A. T., *et al.* (2002) *Nature* **415,** 530–536.
43. Rhodes, D. R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., Barrette, T., Pandey, A. & Chinnaiyan, A. M. (2004) *Neoplasia* **6,** 1–6.
44. Berry, M., Nunez, A.-M. & Chambon, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1218–1222.
45. Musgrove, E. A. & Sutherland, R. L. (1994) *Semin. Cancer Biol.* **5,** 381–389.